

Genotoxic Effects of Low- and High-LET Radiation on Human Epithelial Cells Grown in 2-D versus 3-D Culture

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Risk estimation for radiation-induced cancer relies heavily on human epidemiology data obtained from terrestrial irradiation incidents from sources such as medical and occupational exposures as well as from the atomic bomb survivors. No such data exists for exposures to the types and doses of high-LET radiation that will be encountered during space travel; therefore, risk assessment for space radiation requires the use of data derived from cell culture and animal models. The use of experimental models that most accurately replicate the response of human tissues is critical for precision in risk projections. This work compares the genotoxic effects of radiation on normal human epithelial cells grown in standard 2-D monolayer culture compared to 3-D organotypic co-culture conditions. These 3-D organotypic models mimic the morphological features, differentiation markers, and growth characteristics of fully-differentiated normal human tissue and are reproducible using defined components. Cultures were irradiated with 2 Gy low-LET gamma rays or varying doses of high-LET particle radiation and genotoxic damage was measured using a modified cytokinesis block micronucleus assay. Our results revealed a 2-fold increase in residual damage in 2 Gy gamma irradiated cells grown under organotypic culture conditions compared to monolayer culture. Irradiation with high-LET particle radiation gave similar results, while background levels of damage were comparable under both scenarios. These observations may be related to the phenomenon of "multicellular resistance" where cancer cells grown as 3-D spheroids or *in vivo* exhibit an increased resistance to killing by chemotherapeutic agents compared to the same cells grown in 2-D culture. A variety of factors are likely involved in mediating this process, including increased cell-cell communication, microenvironment influences, and changes in cell cycle kinetics that may promote survival of damaged cells in 3-D culture that would otherwise die or be rendered reproductively inactive in 2-D culture.